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(54) Title: DISINFECTION OF A CONTAMINATED ENVIRONMENT			
(57) Abstract The present invention provides methods and compositions for inhibiting microbial growth through the use of flavonoid aldehydes or alcohols for the purpose of disinfecting contaminated environments. The methods include the step of contacting the unsterile area with an amount of a flavonoid aldehyde or alcohol sufficient to control growth of pathogenic microbes. The aldehyde or alcohol can be provided in a variety of formulations.			

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DISINFECTION OF A CONTAMINATED ENVIRONMENT

INTRODUCTION

5 Technical Field

The present invention involves disinfection of solid, liquid, and gaseous environments using flavonoid aldehydes and alcohols. The invention is exemplified by compositions comprising cinnamic aldehyde and coniferyl aldehyde and their use to decontaminate areas colonized with microorganisms or that are subject to such colonization.

10

Background of the Invention

Most terrestrial environments are colonized by microbes, some of which are potentially infectious pathogens. For example, solid surfaces including walls, floors, food preparation surfaces, and medical instruments often become contaminated by fungal, bacterial, or viral
15 microorganisms. Similarly, such microorganisms often colonize liquids such as the water swimming pools, drinking water, and water used in food processing. Many microorganisms are airborne, traveling from one location to another and possibly spreading disease.

Currently available methods to control or eliminate microbial contamination suffer from several drawbacks. For example, a particular antimicrobial agent generally is effective
20 against only one microbial type. Antibacterial agents generally are ineffective against fungal organisms, and vice versa. Also, antimicrobial agents often are toxic to humans and other animals. Thus, it is of interest to develop compositions and methods that are useful for decontaminating a wide variety of environments.

25 Relevant Literature

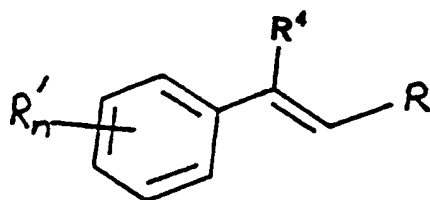
Wolf *et al.*, U.S. Patent No. 4,402,950 reports that cinnamic aldehyde and certain other terpenes obtainable from aromatic plants are useful for deactivating viruses inside living human and animal organisms; Sperti *et al.*, U.S. Patent No. 4,477,361, describe the use of cinnamon compounds in soaps.

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SUMMARY OF THE INVENTION

The present invention provides methods and compositions for inhibiting microbial growth through the use of flavonoid aldehydes or alcohols for the purpose of disinfecting contaminated environments. The methods include the step of contacting the unsterile area with a sufficient amount of a flavonoid aldehyde or alcohol for a sufficient time period to control growth of pathogenic microbes in the unsterile area. The aldehyde or alcohol can be provided in a variety of formulations. Solid surfaces that have been decontaminated using the claimed composition and methods are another aspect of the invention, as are solid surfaces coated with a residue obtained by evaporation of a liquid composition that contains an effective microbial-growth-inhibiting amount of one or more of the flavonoid aldehydes or alcohols.

The biocidal product active ingredient used in the claimed methods and compositions has a structure shown in (1) below



(1)

wherein R represents $-\text{CH}_2\text{OH}$ or $-\text{CHO}$; n is an integer from 0 to 3; and each R_1 independently represents OH or an organic substituent containing from 1 to 10 carbon atoms and from 0 to 5 heteroatoms, wherein the total number of carbon and heteroatoms in all R_1 substituents of said compound is no more than 15; and R_4 represents hydrogen or an organic constituent containing from 1 to 10 carbon atoms. The invention finds use in controlling microbial growth in many environments where disinfection is desirable. The compositions and methods produce environments that are substantially free of microorganisms.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Compositions which include at least one flavonoid aldehyde or alcohol in a formulation suitable for decontaminating an environment and/or producing an environment that is substantially free of microorganisms, and methods of using the compositions are

provided. These compositions include naturally occurring compounds, such as cinnamic aldehyde, coniferyl aldehyde, and closely related compounds. Also of interest are alpha substituted aldehydes, such as alpha hexyl cinnamic aldehyde (HCA). Additionally, the compositions can be used to impregnate organic matter which serves as a nutrient source for
5 a target microorganism and/or can be provided bound to a solid support which itself is non-toxic to animals, including humans. As used herein, "decontaminate" means to disinfect or sterilize an environment that is colonized by microorganisms, or inhibit the growth of microorganisms in the environment so as to decrease the size of the microorganism population in a given area. Such decontamination can occur immediately upon application of
10 the biocidal agent, or can be the result of a residual effect of the compositions for maintaining the cleanliness of areas susceptible to microbial contamination and to prevent such areas from becoming contaminated. The environment can be liquid, gas or solid.

Compositions comprising compounds according to formula (1) have several advantages as disinfectants. Unlike most other antimicrobial compositions, they are effective
15 against both bacterial and fungal microbes, and also are effective against viruses. Another advantage of compounds of formula (1) is that they are nontoxic to humans and animals; a number of the aromatic and aliphatic aldehydes which may find use in the subject invention, such as alpha hexyl cinnamaldehyde (HCA), benzaldehyde, acetaldehyde, cinnamaldehyde, piperonal, and vanillin are generally regarded as safe (GRAS) synthetic flavoring agents (21
20 CFR §172.515). HCA was in public use before the 1950's and today is widely used in consumer preparations (soaps, detergents, creams, lotions, perfumes) (Monographs on fragrances raw materials. Food Cosmet. Toxicol. 12: suppl., 915, 1974). HCA was granted GRAS (generally recognized as safe) status by FEMA (Flavoring Extract Manufacturers' Association. Survey of flavoring ingredient usage levels. No. 2569. Fd. Technol.,
25 Champaign, 19: (part 2) 155, 1965) in 1965 and is approved by the US FDA for use in food (21CFR121.1164). The Council of Europe (1970) (Council of Europe. Natural and Artificial Flavoring Substances. Partial Agreement in the Social and Public Health Field. Strasbourg, List A(1), Series 1, no. 129, p. 55, 1970) included HCA in the list of admissible artificial flavoring substances at a level of 1 ppm. Various of these compounds have been
30 reported to have inhibitory activity against *C. botulinum* spore germination. Bowles and Miller, *G. Food Protection* (1993) 56: 788-794. Another advantage is that the compositions can be used in and/or around food sources. The aromatic aldehydes in particular have positive organoleptic and olfactory properties which in some cases may improve the flavor

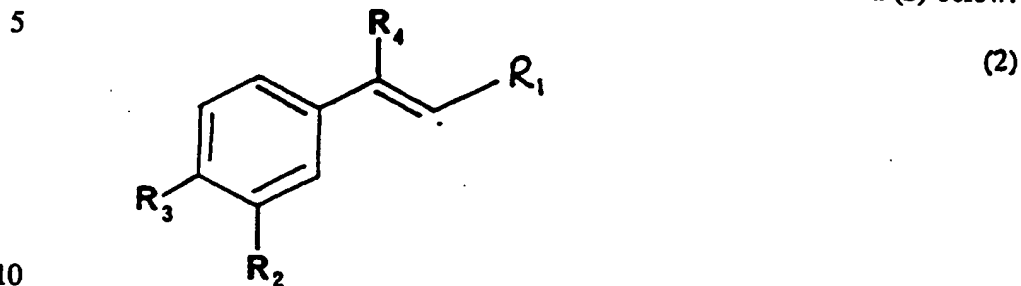
and/or smell of treated products. The odor of HCA for example is described as floral or jasmine-like with some herbaceous character (Technical Data Sheet).

Other advantages are that the compositions can be formulated as aqueous emulsions with surfactants and other compounds, such as the Tweens (polysorbates) and saponin (which also has GRAS status) and are used as food additives. The safety of the formulations therefore is already assured when used within the approved concentrations. Additional advantages include that the formulation residuality can be managed. This is of great benefit when short term residuals are desired for integrated decontamination management programs with beneficial organisms. In addition, the formulations work against microorganisms which are resistant to other agents and are effective on multiple target organisms. Examples of such organisms include fungi, bacteria (*e.g.*, *Salmonella*, *C. botulinum*, *M. tuberculosis*, etc.), protozoa (*e.g.*, *Giardia*), viruses, and algae, which includes organisms that are pathogenic to humans or other animals. This reduces the need for application of multiple agents to the environment of interest to control all microorganisms in a particular area. The effects of a single application of the formulation are long lasting, and generally a single application is sufficient to control microbial growth for at least a month or longer. The long term control of pathogenic organisms results in a healthier environment; the lower concentrations and single dose of antipathogenic agents generally necessary to decontaminate an area decrease the likelihood of damage to the environment as well as decrease the likelihood of any adverse side effects to workers applying the compounds, or to animals, fish or fowl which may come into contact with treated materials. Typically the formulations are rapidly lethal to a target organism.

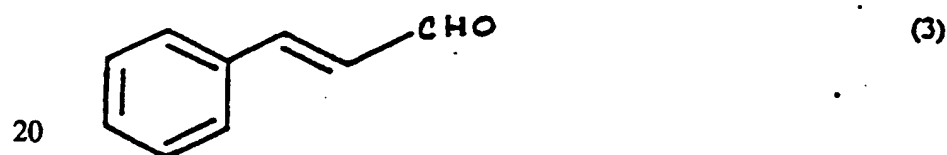
When applied to animals, including humans, HCA is non-toxic and non-irritating to the skin at the concentrations used. For example, α -hexyl cinnamaldehyde (HCA) has an oral LD₅₀ of 3.1 g/kg in rats and a dermal LD₅₀ of greater than 3 g/kg (Moreno, O.M. Report to RIFM, March 24, 1971). HCA was found to be moderately irritating when the neat compound was applied to intact or abraded rabbit skin for 24 hours under occlusion (Moreno). When tested at 12% in petrolatum, HCA produced no irritation after a 48 hour closed-patch test on human subjects and produced no sensitization in a maximization test carried out on 25 human subjects (Kligman (1966) *J. Invest. Dermatol.* 47: 393). HCA at 20% in diethylphthalate produced no positive reactions in a repeated insult patch test conducted on 100 human subjects. In studies using the maximization test in guinea pigs, Senma and coworkers report a tendency that as the number of hydrocarbons of alkyl groups

replacing the alpha-hydrogen in cinnamaldehyde increased, the rate of sensitization reaction declined.

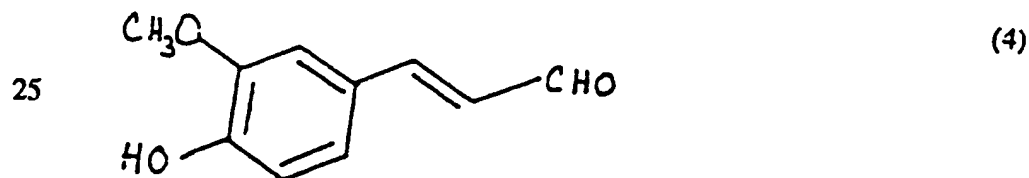
The biocidal ingredients used in the claimed compositions and methods are flavonoid aldehydes or alcohols. A preferred compound is shown in formula (2) below:



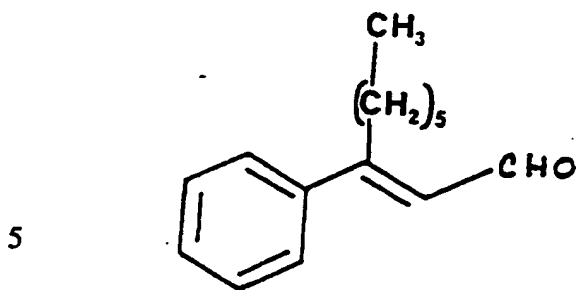
wherein R₁ represents-CHO, R₂ represents-OH or an organic substituent containing from 1 to 10 carbon atoms, R₃ represents a methoxy group or organic substituent containing from 1 to 10 carbon atoms, and R₄ represents a hydrogen or an organic substituent containing from 1 to 10 carbon atoms. Of particular interest are flavonoid aldehydes, particularly aromatic aldehydes. Examples of aromatic aldehydes of use in the present invention are cinnamic aldehyde ((3) below):



and coniferyl aldehyde ((4) below).



Other compounds of interest include analogs of the compound of formula (1) such as compounds substituted at the alpha position with an alkyl, such as a hexyl group, or a branched alkyl group such as an amyl group. Generally the group at the alpha position is from C-5 to C-10. Such compounds include α -hexyl cinnamaldehyde and α -amyl cinnamaldehyde. The chemical structure of α -hexyl cinnamic aldehyde (HCA) is shown in (5) below.



The Chemical Abstracts Service (CAS) name of HCA is 2-(phenylmethylene) octanal and the CAS Registry Number is [101-86-0]. The compound is also described by the chemical name of 2-hexyl-3-phenyl-2-propenal. The compound's formula is $C_{15}H_{20}O$ and molecular weight is 216.3. HCA can be obtained from Firmenich; their product is composed principally of the (E)-cis isomer (93.8% maximum), and the (Z)-trans isomer (6% maximum). Among minor components is the self aldol condensation product of octanal (1-1.5% (Personal Communication, June Burkhardt, Firmenich, Plainsboro, New Jersey).

In addition to the specific compounds of the formulas (1), (2), (3) (4) and (5) set forth above, derivatives of any of these compounds that produce a compound of the formula identified above upon action of a biological system on precursor are considered to be equivalent to compounds of the invention. Thus application of precursor compounds to microorganisms that can metabolize the precursors to produce a specific compound of formula (1), (2), (3), (4) or (5) would be equivalent to the practice of the present invention. Biological conversion of precursor compounds into flavonoid aldehydes is described in, for example, U.S. Patent No. 5,149,715 and references cited therein. See also Casey and Dobb, *Enzyme Microb. Technol.* (1992) 14: 739-747.

HCA can be synthesized as described, for example, in USPN 5,055,621. On a laboratory scale, HCA can be synthesized by reaction of benzaldehyde with octanal under a nitrogen atmosphere (aldol condensation). The reaction is conducted in a stirred flask charged with methanol, 309 ppm diphenylamine, potassium hydroxide and benzaldehyde. Following the slow addition of octanal, the reaction mixture is brought to a pH of 7.5-9.5 with acetic acid. Following evaporation of methanol and wash of the reaction mixture with water, the organic phase is transferred to a distillation unit. Approximately 20-24% of the pot charge is removed as benzaldehyde and "lights", with the remaining distillate constituting alpha-hexylcinnamic aldehyde "heart cut." The "heart cut" is subjected to an additional fractionation, in which 1-5% (by weight) of the material may be removed in "light" fractions, depending upon odor evaluation. The final product is a light yellow oil having a specific

gravity of 0.955-0.965 at 20°C, a refractive index of 1.548-1.562 at 20°C, a boiling point of 305°C at 1 atmosphere, and a melting point of 26°C. The commercial product (Firmenich Chemical Manufacturing Center) is stabilized with the addition of 0.04% 2, 6-di-tert-butyl-p-cresol (butylated hydroxytoluene or BHT), which serves as an anti-oxidant (Technical Data Sheet, Hexylcinnamic aldehyde 907600, Revision 853, Firmenich Inc., Plainsboro, New Jersey). HCA can also be isolated from rice in which it occurs naturally. (Givaudan-Roure Index, Givaudan-Roure Corporation, Clifton, New Jersey, 1994, p. 89).

The compositions typically comprise biocidal agents that can be used alone in a suitable diluent for the environment of interest or can be prepared as formulations that facilitate application of the biocidal agents to the environment to be decontaminated. The biocidal compounds are typically in the form of concentrated liquids, solutions, suspensions, powders and the like, containing such concentration of the biocidally active compound as is suited for a particular purpose. The compositions can be applied directly to an area susceptible to colonization by microbial organisms in the form of solution in a suitable solvent; the choice of solvent is dictated in some cases by the nature of the environment to be treated, and can be chosen based upon the type of solvent generally used for application to such an environment. As an example, where the surface to be decontaminated is for use with food stuffs, and long term effects are desirable, it is important that any residue from the composition is non-toxic to humans or other consumers of the food stuffs. Where the use is one where only a short term effect is desired and/or no residue, a volatile organic solvent may be preferable, particularly where there is no likelihood of ingestion of the formulation.

The biocidal ingredient is present in the formulations in an effective microbial growth-inhibiting amount. An "effective microbial growth-inhibiting amount" is that amount of biocidal ingredient that is effective in inhibiting the growth of microorganisms that are present in the environment or in preventing microorganisms from becoming established in the environment. Growth of a population of microorganisms is said to be "inhibited" if the microorganisms in the particular environment multiply at a slower rate in the presence of the biocidal agent than in an equivalent environment that is not treated with the biocidal agent. Preferably, the biocidal agent will result in the microorganisms multiplying at a rate that is less than about 70% of that observed for microorganisms in an untreated environment. More preferably, the rate of multiplication for microorganisms in a treated environment will be less than about 50%, and most preferably less than about 30% of that observed for microorganisms in an untreated environment. An effective microbial growth-inhibiting

amount of the flavonoid aldehyde or alcohol, when formulated as described herein, generally is about 0.01 to 5.0 weight percent. More preferably, the concentration of the biocidal agent is about 0.1 to 2.5 weight percent.

5 The biocidal aldehyde or alcohol compounds can be used either alone or in combination with other active or inactive substances. Where the formulation is an aqueous solution, it optionally can contain a salt of a polyprotic acid, such as sodium bicarbonate, sodium sulfate, sodium phosphate or sodium biphosphate. An emulsifier such as Tween 80 or saponin also can be included in the formulation as appropriate to aid in preparation of the formulation and/or to increase the biocidal properties of the formulation or to provide
10 substantive properties. For use as a means of cleansing a surface, although the biocidal compound can be formulated as a soap or a detergent. Suitable detergents for the formulation include anionic detergents such as those described in U.S. Patent No. 4,978,686. The resulting emulsion is diluted to an appropriate concentration for use, and is additionally provided in a formulation suitable for the intended application, for example, as a household
15 cleaner, carpet shampoo, detergent, or animal dip shampoo or soap. Generally, it is unnecessary to include antioxidants such as vitamin E, eugenol, BHT, n-propyl gallate and the like, in the formulation, particularly where the flavonoid compound used itself has antioxidant properties, for example conferyl aldehyde.

The formulations utilized in the claimed compositions and methods are typically free
20 of substantive agents such as free fatty acids or emollients. For example, the formulations preferably contain less than about 5% by weight free fatty acids such as coconut fatty acid, tallow fatty acid, stearic acid, and oleic acid, as well as fatty acid ester soaps that contain free fatty acids or emollients such as glyceryl monostearate, hexadecyl stearate, diethyleneglycol dioleate, and the like. The formulations also generally contain less than
25 about 5% weight percent of the emollients isopropyl myristate, isopropyl palmitate, isopropyl stearate, hexadecyl stearate, dihexadecyl adipate, and butyl stearate.

Stability of the formulation can be evaluated by a variety of methods, including accelerated tests in which a formulation of interest is exposed to elevated temperatures over a set time. Samples of the formulations are taken at regular intervals and analyzed chemically
30 by methods known to those skilled in the art to determine the rate and nature of degradation. For example, HCA can be analyzed by Gas Liquid Chromatography (GLC), using a 30 meter non-polar polydimethylsiloxane capillary column (e.g. HP-1, Hewlett-Packard, or SPB-1, Supelco) and a flame-ionization detector. Using helium as a carrier gas (8 ml/min.) and a

column temperature of approximately 240°C, the (E)-cis isomer (major component) has a retention time of approximately 6.0 minutes and the (Z)-trans isomer (minor component) has a retention time of approximately 6.3 minutes.

For some applications, the compounds can be bound to a solid support, either prior to or upon application to the environment that is to be decontaminated. For example, the biocidal compounds can be coupled to a solid support for application in powder or granular form, such as microcrystalline cellulose or bound to paper or other material suitable for lining drawers or cupboards susceptible to contamination, for example, from insect or rodent droppings, and the like. Typically, the biocidal aldehydes and alcohols are coupled to the solid support by means of a bifunctional linker that has a moiety that is reactive with the aldehyde or alcohol and another moiety, such as a binding domain that can be derived from a polysaccharide and binds to the solid support. The biocidal aldehyde or alcohol is bound to the binding domain with or without a cleavable bond. The preparation and use of polysaccharidase binding domains is described in U.S. Patent Nos. 5,340,731; 5,202,247 and 5,166,317. Where a solid carrier is used, the coupling reaction should avoid materials that can lead to oxidation of the active aldehydes. Examples of solid delivery systems include starch-dextran and the like. See, e.g., Yuan *et al.*, *Fundamental and Applied Toxicology* (1993) 20: 83-87, for examples of delivery systems.

The compositions can be applied as a conjugate between the flavonoid and the binding domain to a surface to which the binding domain binds. When applied to that surface, the biocidal agent is bound to the surface. Examples of such surfaces include cellulose such as paper or wood. The surface to which the conjugate is applied can be either clean or already colonized by microbes.

In use, an environment is contacted with an effective microbial-growth-inhibiting amount of a formulation comprising one or more of the compounds shown in (1) above. The particular method used to "contact" an environment with the biocidal composition is dependent at least in part upon the nature of the environment. Environments for which the claimed compositions and methods are useful include solids, gases, and liquids that are contaminated with microorganisms, or are susceptible to becoming contaminated. Decontamination of solids using the formulation of the invention are influenced by the degree of porosity of the target environment. Generally, the degree of porosity depends on pore size, uniformity and occurrence, which can vary substantially for any given solid. Porosity generally affects the nature of contact between the biocidal agent and the target organism.

Likewise, decontamination of liquids and gases are influenced by contact of the antimicrobial composition with a target microorganism. Thus, the method and formulation used for decontamination of an environment of interest is adapted for a particular purpose at hand, with particular consideration given the nature of contact, such as duration of exposure
5 between the biocide and the target organism of interest and the concentration of the biocide in the formulation.

Of particular interest is the use of the compositions and methods of the invention for decontamination in the health fields. The compositions and methods of the invention find use in treatment of generally porous components (*i.e.*, adsorbents) such as surgical clothing,
10 sponges, dressings, wipes, bandages, and incontinence products, generally non-porous surfaces (*i.e.*, non-adsorbents) such as walls, floors, work surfaces, vessels, baths, sinks as well as sanitaryware and tools of metal, plastics, ceramics or glass, wood and rubber among others; decontamination of liquids such as drinking water, waste water and biological fluids and gases such as contaminated hospital air.

15 Decontamination of a nonporous and porous solid environments can be accomplished by contacting the solid with a formulation of the invention by any number of means known in the art, such as dipping, spraying, immersing or washing among others. Decontamination of gases and/or liquids can be accomplished by admixing with formulations of the invention to permit sufficient contact of a target organism with an effective amount of the biocidal
20 formulation. Treatment of gases and liquids can be accomplished also by passing them through a solid impregnated with a formulation of the invention.

Additionally of interest is the decontamination of porous solids by using the compositions of the invention as components of the solid. For this application, it is necessary to impregnate a formulation of the invention with a porous solid of interest, by absorption,
25 adsorption, covalent or non-covalent means before and/or after contamination. This can be accomplished, for example, through permeation of the solid with the formulation and/or providing a porous solid containing a means for covalent or non-covalent binding of a formulation to the solid. In a preferred embodiment, the porous solid contains cellulose and the formulation of the invention is linked to a cellulose binding domain, thus providing a
30 means for attaching the flavonoid aldehyde to the solid. A particular application of interest is use of the formulations as components of generally porous medical provisions and incontinence products.

Using the above methods and compositions of the invention, gases, liquids, and solids are provided which are substantially free of pathogenic organisms. The methods and compositions of the invention are thus generally applicable to food collection processes, preparation, serving environments, materials and facilities as well as other contact sensitive areas such as day and child care facilities, medical facilities, nursing homes and other health care facilities, domestic households and various food processing facilities.

The following examples are offered by way of illustration and not by way of limitation.

EXAMPLES

10 All experiments run with test formulations include a positive active ingredient control and a negative formulation-without-active-ingredient control. The test formulations are run over a concentration range of at least two log units to give sufficient data to calculate an inhibitory concentration at 50% of control (IC50).

15 Example 1

The antipathogenic effect of a particular formulation on a given pathogen is measured for each formula and component with or without a serial diluent of any additional component of interest, and optimal dose-range for a given application calculated. Components of a formulation for a particular application are determined as follows. A dose response curve for a given formulation is generated by evaluating first the concentration range over which a given component of formula (1) has no activity to maximum activity against a test organism. For this purpose, serial dilutions of a test formulation of a flavonoid aldehyde in a concentration of 0.001 and 10.0 weight percent is prepared. A secondary dose response curve is generated to evaluate the component separately and in combination with other components including surfactant, carrier and/or adjuvant. Activity is measured by standard means to determine the biocidal effect of a formulation against one or more target microorganisms. IC50 is calculated for each organism and formulation tested. Substantiveness of the formulation for a given application is determined by calculating IC50 through separate and combined application of components of formula (1) with surfactant, carrier and/or adjuvant. Efficacy of a given formulation is tested by increasing or decreasing doses, concentrations and application methods of the formulation.

Surface Sterilization

Decontamination of stainless-steel, tile, wood, plastic, and/or rubber surfaces are tested. See, *e.g.*, applicable sections of the Association of Official Analytical Chemists, 15th edition, 1990 Environmental Protection Guidelines, United States of America.

5

Water Treatment

Decontamination of waste water from chicken-processing is tested. Preservation of stored water is tested. See, *e.g.*, applicable sections of the Association of Official Analytical Chemists, 15th edition, 1990 Environmental Protection Guidelines, United States of America.

10

Disinfection of Air

Decontamination of contaminated air is tested. See, *e.g.*, applicable sections of the Association of Official Analytical Chemists, 15th edition, 1990 Environmental Protection Guidelines, United States of America.

15

Example 2

Activity of cinnamic aldehyde, coniferyl aldehyde, and/or alpha hexyl cinnamaldehyde against mold and mildew is tested as in Example 1.

20

Example 3

Activity of cinnamic aldehyde, coniferyl aldehyde and/or alpha hexyl cinnamaldehyde against influenza A virus is tested as in Example 1.

25

Example 4

Activity of cinnamic aldehyde, coniferyl aldehyde, and/or alpha hexyl cinnamaldehyde against protozoa or algae is tested as in Example 1 using minimum inhibitory concentration (MIC) and dose response against *Giardia* and swimming pool algae.

30

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. All publications and patent documents referenced in this application are incorporated herein by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims.

CLAIMS

1. A method for decontaminating an environment, said method comprising:
contacting said environment with an effective microbial-growth-inhibiting amount of one or
more compounds of structure (2) formulated in a formulation substantially free of fatty acids
and emollients.
2. The method according to Claim 1, wherein said environment is a solid surface.
3. The method according to Claim 2, wherein said solid surface is non-porous.
4. The method according to Claim 3, wherein said solid surface comprises a food
preparation surface.
5. The method according to Claim 3, wherein said solid surface comprises a
medical surface.
6. The method according to Claim 1, wherein said environment is a fluid.
7. The method according to Claim 3, wherein said fluid is water applied to
foodstuffs during processing.
8. The method according to Claim 7, wherein said contacting comprises
dispersing in and said effective microbial-growth-inhibiting amount is about 0.01 and 5.0
weight percent.
9. The method according to Claim 3, wherein said fluid is stored water.
10. The method according to Claim 9, wherein said stored water is potable.
11. The method according to Claim 9, wherein said stored water is contained in a
swimming pool.
12. The method according to Claim 1, wherein said environment is a gas and said
formulation is reversibly bound to a solid support.
13. The method according to Claim 12, wherein said gas is air.
14. The method according to Claim 12, wherein said solid support is cellulose.
15. A method for cold surface sterilization, said method comprising:
contacting a solid surface with an effective microbial-growth-inhibiting amount of a
formulation comprising about 0.01 and 5.0 percent by weight of one or more compounds of
structure (2) and substantially free of fatty acids and emollients.
16. The method according to Claim 15, wherein said contacting is selected from
the group consisting of immersing, dipping, washing and spraying and said formulation is a
liquid and said solid surface is a medical surface.

17. The method according to Claim 15, wherein said contacting is selected from the group consisting of wiping, washing and spraying and said formulation is a liquid and said solid surface is a food processing surface.

18. A method for disinfecting, said method comprising:
5 contacting a fluid with an effective microbial-growth-inhibiting amount of a formulation comprising 0.01 to 5.0 percent by weight of one or more compounds of structure (2) and substantially free of fatty acids and emollients.

19. The method according to Claim 18, wherein said contacting is selected from the group consisting of dispersing in, admixing in and treating with and said fluid is stored
10 water.

20. The method according to Claim 18, wherein said contacting is selected from the group consisting of dispersing in, admixing in and treating with and said fluid is water involved in food processing.

21. A method for disinfecting, said method comprising:
15 filtering air through a solid support associated with an effective microbial-growth-inhibiting amount of a formulation comprising 0.01 to 5% percent by weight of one or more compounds of structure (2).

22. The method according to Claim 21, wherein said solid support is comprised of cellulose.

20 23. The method according to Claim 21, wherein said associated is reversibly bound.

24. A composition comprising:
0.01 to 5.0 percent by weight of one or more compounds of formula (2) in a formulation substantially free of fatty acids and emollients and suitable for producing an
25 environment substantially free of microorganisms.

25. The composition according to Claim 24, wherein said one or more compounds are of formula (3) or formula (4).

26. The composition according to Claim 25, wherein said composition is an aqueous emulsion.

30 27. The composition according to Claim 25, wherein said formulation further comprises at least one additive selected from the group consisting of a salt of a mineral acid and an emulsifier.

28. The composition according to Claim 27, wherein said salt is sodium bicarbonate.

29. The composition according to Claim 27, wherein said emulsifier is Tween 80.

30. The composition according to Claim 24, wherein said formulation is a
5 dispersable liquid.

31. The composition according to Claim 24, wherein said formulation is associated with a solid support.

32. The composition according to Claim 31, wherein said solid support is comprised of cellulose.

10 33. A substantially non-porous solid surface decontaminated according to the method of Claim 1.

34. A solid surface coated with a residue obtained by evaporation of a liquid composition comprising an effective microbial-growth-inhibiting amount of one or more compounds of formula (2).

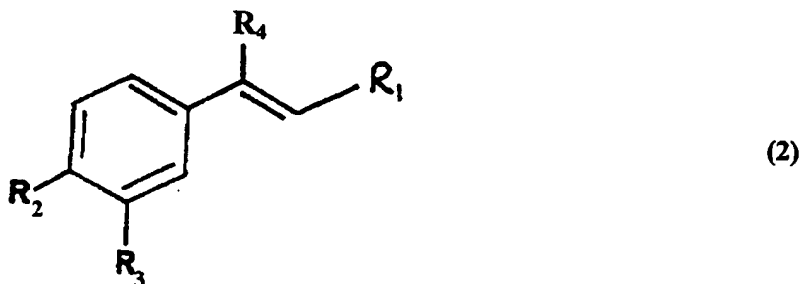
15 35. The solid surface according to Claim 34, wherein said residue has a non-greasy texture.

36. The solid surface according to Claim 34, wherein said effective microbial-growth-inhibiting amount comprises 0.1 to 5.0 percent by weight of said residue.

[received by the International Bureau on 7 October 1996 (07.10.96);
original claims 1-36 replaced by new claims 1-38 (8 pages)]

1. A method for decontaminating a non-porous solid surface, said method comprising:

5 contacting said solid surface with a composition comprising an effective microbial-growth-inhibiting amount of one or more compounds having the formula

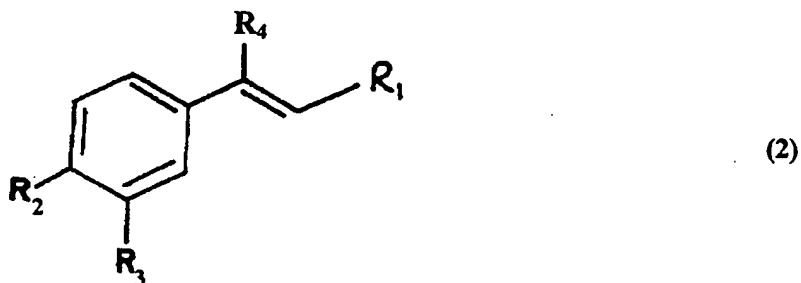


wherein R_1 represents -CHO, R_2 represents -H, -OH or an organic substituent containing from 1 to 10 carbon atoms, R_3 represents -H, a methoxy group or organic substituent containing from 1 to 10 carbon atoms, and R_4 represents a hydrogen or an organic substituent containing from 1 to 10 carbon atoms; provided that said composition is substantially free of fatty acids and emollients.

2. The method according to Claim 1, wherein said solid surface comprises a food preparation surface.

3. The method according to Claim 1, wherein said solid surface comprises a medical surface.

4. A method for decontaminating a fluid, said method comprising:
contacting said fluid with a composition comprising an effective microbial-growth-inhibiting amount of one or more compounds having the formula



wherein R_1 represents -CHO, R_2 represents -H, -OH or an organic substituent containing from 1 to 10 carbon atoms, R_3 represents -H, a methoxy group or organic substituent containing from 1 to 10 carbon atoms, and R_4 represents a hydrogen or an organic substituent containing from 1 to 10 carbon atoms; provided that said composition is substantially free of fatty acids and emollients.

5 5. The method according to Claim 4, wherein said fluid is water applied to foodstuffs during processing.

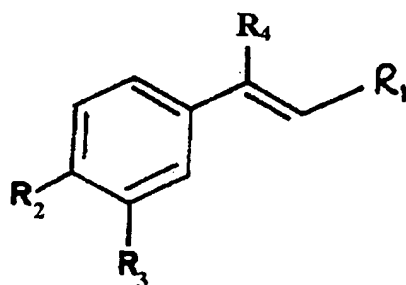
6. The method according to Claim 5, wherein said contacting comprises dispersing in said fluid, and said effective microbial-growth-inhibiting amount is about 0.01
10 to about 5.0 weight percent.

7. The method according to Claim 4, wherein said fluid is stored water.

8. The method according to Claim 7, wherein said stored water is potable.

9. The method according to Claim 7, wherein said stored water is contained in a swimming pool.

15 10. A method for decontaminating a gas, said method comprising:
contacting said gas with a composition comprising an effective microbial-growth-inhibiting amount of one or more compounds having the formula



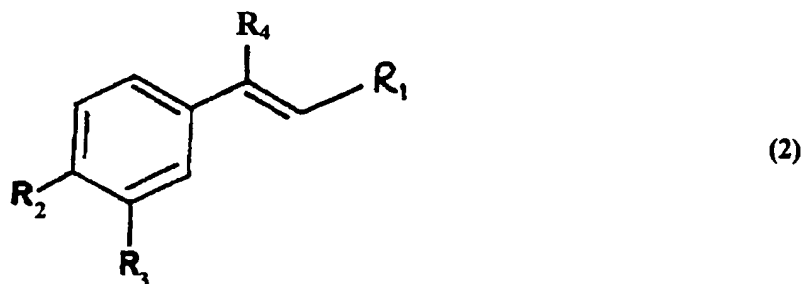
(2)

20 wherein R_1 represents -CHO, R_2 represents -H, -OH or an organic substituent containing from 1 to 10 carbon atoms, R_3 represents -H, a methoxy group or organic substituent containing from 1 to 10 carbon atoms, and R_4 represents a hydrogen or an organic substituent containing from 1 to 10 carbon atoms; provided that said composition is substantially free of fatty acids and emollients; further provided that said composition is bound to a solid support.

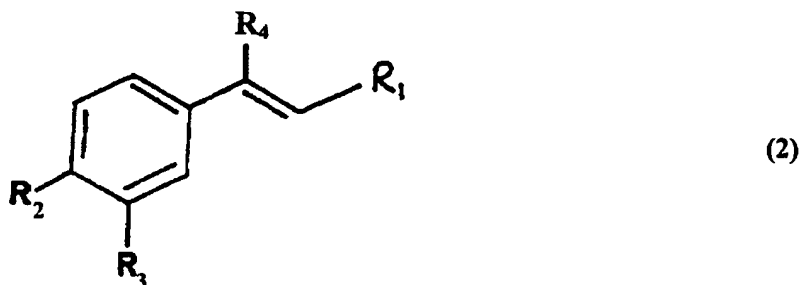
25 11. The method according to Claim 10, wherein said gas is air.

12. The method according to Claim 10, wherein said solid support is cellulose.

13. A method for cold surface sterilization, said method comprising:
contacting said surface with an effective microbial-growth-inhibiting amount of a formulation
comprising 0.01 to 5.0 percent by weight of one or more compounds having the formula



- 5 wherein R_1 represents -CHO, R_2 represents -H, -OH or an organic substituent containing from 1 to 10 carbon atoms, R_3 represents -H, a methoxy group or organic substituent containing from 1 to 10 carbon atoms, and R_4 represents a hydrogen or an organic substituent containing from 1 to 10 carbon atoms; provided that said composition is substantially free of fatty acids and emollients.
- 10 14. The method according to Claim 13, wherein said contacting is selected from the group consisting of immersing, dipping, washing and spraying and said formulation is a liquid and said solid surface is a medical surface.
- 15 15. The method according to Claim 13, wherein said contacting is selected from the group consisting of wiping, washing and spraying and said formulation is a liquid and said solid surface is a food processing surface.
16. A method for disinfecting, said method comprising:
contacting a fluid with an effective microbial-growth-inhibiting amount of a formulation
comprising 0.01 to 5.0 percent by weight of one or more compounds having the formula

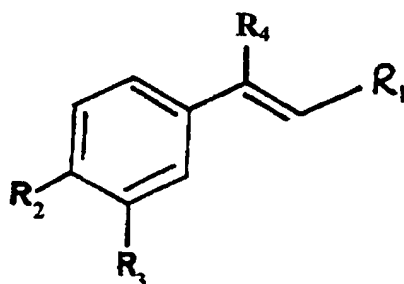


wherein R_1 represents -CHO, R_2 represents -H, -OH or an organic substituent containing from 1 to 10 carbon atoms, R_3 represents -H, a methoxy group or organic substituent containing from 1 to 10 carbon atoms, and R_4 represents a hydrogen or an organic substituent containing from 1 to 10 carbon atoms; provided that said composition is substantially free of fatty acids and emollients.

17. The method according to Claim 16, wherein said contacting is selected from the group consisting of dispersing in, admixing in and treating with and said fluid is stored water.

18. The method according to Claim 16, wherein said contacting is selected from the group consisting of dispersing in, admixing in and treating with and said fluid is water involved in food processing.

19. A method for disinfecting, said method comprising:
filtering air through a solid support associated with an effective microbial-growth-inhibiting amount of a formulation comprising 0.01 to 5% percent by weight of one or more compounds having the formula



(2)

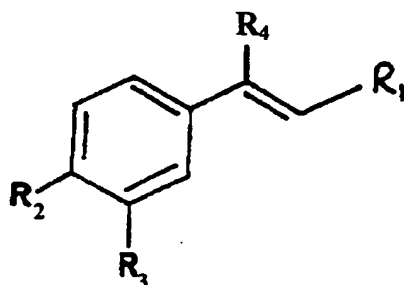
wherein R_1 represents -CHO, R_2 represents -H, -OH or an organic substituent containing from 1 to 10 carbon atoms, R_3 represents -H, a methoxy group or organic substituent containing from 1 to 10 carbon atoms, and R_4 represents a hydrogen or an organic substituent containing from 1 to 10 carbon atoms; provided that said composition is substantially free of fatty acids and emollients.

20. The method according to Claim 19, wherein said solid support comprises cellulose.

21. The method according to Claim 19, wherein said associated is reversibly bound.

22. A composition suitable for producing an environment substantially free of microorganisms comprising:

0.01 to 5.0 percent by weight of one or more compounds having the formula



(2)

5 wherein R₁ represents -CHO, R₂ represents -H, -OH or an organic substituent containing from 1 to 10 carbon atoms, R₃ represents -H, a methoxy group or organic substituent containing from 1 to 10 carbon atoms, and R₄ represents a hydrogen or an organic substituent containing from 1 to 10 carbon atoms; provided that said composition is substantially free of
10 fatty acids and emollients.

23. The composition according to Claim 22, wherein said one or more compounds are selected from the group consisting of cinnamic aldehyde, coniferyl aldehyde and alpha-hexyl cinnamaldehyde.

24. The composition according to Claim 23, wherein said composition is an
15 aqueous emulsion.

25. The composition according to Claim 23, wherein said composition further comprises at least one additive selected from the group consisting of a salt of a mineral acid and an emulsifier.

26. The composition according to Claim 25, wherein said salt is sodium
20 bicarbonate.

27. The composition according to Claim 25, wherein said emulsifier is Tween 80.

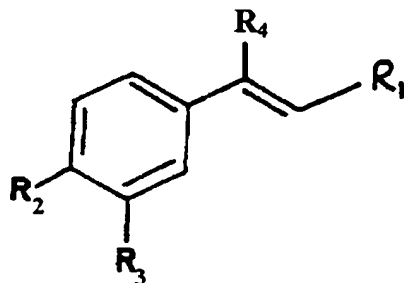
28. The composition according to Claim 22, wherein said composition is a dispersable liquid.

29. The composition according to Claim 22, wherein said composition is
25 associated with a solid support.

30. The composition according to Claim 29, wherein said solid support comprises cellulose.

31. A composition comprising:

5 a non-porous solid surface coated with a residue obtained by evaporation from said surface of a liquid composition comprising an effective microbial growth-inhibiting amount of one or more compounds having the formula

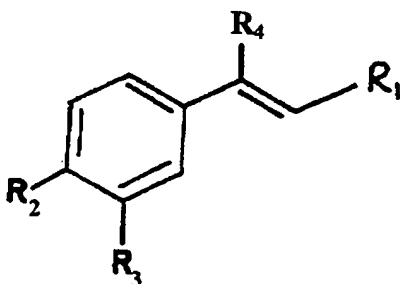


(2)

10 wherein R₁ represents -CHO, R₂ represents -H, -OH or an organic substituent containing from 1 to 10 carbon atoms, R₃ represents -H, a methoxy group or organic substituent containing from 1 to 10 carbon atoms, and R₄ represents a hydrogen or an organic substituent containing from 1 to 10 carbon atoms; provided that said composition is substantially free of fatty acids and emollients.

32. A composition comprising:

15 a porous solid surface coated with a residue obtained by evaporation from said surface of a liquid composition comprising an effective microbial growth-inhibiting amount of one or more compounds having the formula



(2)

wherein R_1 represents -CHO, R_2 represents -H, -OH or an organic substituent containing from 1 to 10 carbon atoms, R_3 represents -H, a methoxy group or organic substituent containing from 1 to 10 carbon atoms; and R_4 represents a hydrogen or an organic substituent containing from 1 to 10 carbon atoms; provided that said liquid composition is substantially free of fatty acids and emollients; further provided that said effective microbial growth-inhibiting amount comprises 0.1 to 5.0 percent by weight of said residue.

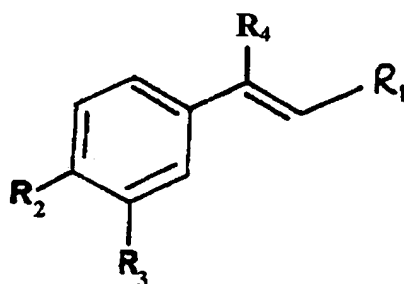
33. The composition according to claim 32, wherein said solid is selected from the group consisting of surgical clothing, sponges, dressings, wipes, bandages and incontinence products.

34. The composition according to claim 32, wherein said solid comprises cellulose.

35. The composition according to claim 34, wherein said compound is attached to said cellulose via a cellulose binding domain.

36. The composition according to claims 31 or 32, wherein said compounds are selected from the group consisting of cinnamic aldehyde, coniferyl aldehyde and alpha-hexyl cinnamaldehyde.

37. A method for decontaminating a fluid or a gas, said method comprising : passing said fluid or gas through a solid impregnated with a formulation comprising an effective microbial growth-inhibiting amount of one or more compounds having the formula



wherein R_1 represents -CHO, R_2 represents -H, -OH or an organic substituent containing from 1 to 10 carbon atoms, R_3 represents -H, a methoxy group or organic substituent containing from 1 to 10 carbon atoms, and R_4 represents a hydrogen or an organic substituent containing from 1 to 10 carbon atoms; provided that said composition is substantially free of fatty acids and emollients; whereby said fluid or gas is decontaminated.

38. The method according to any one of claims 1-21, and 37 wherein said compounds are selected from the group consisting of cinnamic aldehyde, coniferyl aldehyde and alpha-hexyl cinnamaldehyde.

INTERNATIONAL SEARCH REPORT

International Application No

PL/US 95/17008

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A01N35/02 A61L9/01

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A01N A61L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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X	GB,A,1 060 447 (MAPLE LEAF TRUST) 1 March 1967 see page 1, line 45 - line 60; claims ---	1-14
X	GB,A,472 623 (H. BECHHOLD) 24 December 1935 see page 5, line 105 - line 115; claims ---	1,12,13
X	PATENT ABSTRACTS OF JAPAN vol. 017, no. 475 (C-1103), 30 August 1993 & JP,A,05 117125 (DAINICHISEIKA COLOR & CHEM MFG CO LTD), 14 May 1993, see abstract --- -/-	1,15-17

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

23 July 1996

Date of mailing of the international search report

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 95/17008

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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X	PATENT ABSTRACTS OF JAPAN vol. 011, no. 066 (P-552), 27 February 1987 & JP,A,61 228412 (NIPPON KOGAKU KK), 11 October 1986, see abstract ---	1
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International Application No

PCT/US 95/17008

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